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Effect of elevated carbon dioxide and rooting hormone on propagation of *Euonymus* 'Moonshadow'

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Abstract

Although effect of rooting hormone in the propagation of ornamental species has been studied by many scientists, very few have contributed to understanding the effect of supplemental carbon dioxide(CO_2) in rooting of plants. With the aim of understanding the effect of CO_2 in rooting and its interaction with rooting hormone treatment, a greenhouse study was conducted. Two identical greenhouses were used in which, one was supplied with an average of 800 ppm of $CO_2$and the other one was at about 400 ppm (ambient) throughout the rooting period. Rooting hormone treatments were control, 1000, 3000 and 5000 ppm concentrations of Dip'N Grow. Elevated CO_2 did not promote root development when compared to ambient condition. However, increasing rooting hormone concentration significantly affected the rooting parameters. Rooting percentage was not improved under elevated CO_2. Ambient CO_2 with 5000 ppm concentration of Dip'N Grow showed greater root number.

Key words: Dip 'N Grow, winter creeper, supplemental $CO_{2,}$ cuttings

Introduction

Propagation of ornamental shrubs is an important aspect of the nursery and ornamental industry. Among different methods of asexual propagation, taking cutting is a widely used method for cloning shrubs and trees (Witcher *et al*., 2014). Cutting propagation is important in tree and shrub improvement programs to reduce production time, helps in mass vegetative production, and ensures the establishment of clonal seed orchard (Kesari *et al*., 2009). Furthermore, use of cuttings eliminates +g of root initiation for early production of cuttings are some reported advantages of CO_2 supplementation during propagation (Rogers *et al*., 1999).

Euonymus fortunei(Turcz.) Hand.-Maz. 'Moonshadow' commonly known as the spindle, Fortunei's spindle, winter creeper, or wintercreeper is a bushy shrub belonging to the family Celastraceae and is native to East Asia. The genus consists of more than 176 species and varieties that are mostly evergreen shrubs and have landscape importance (Hou, 1975). The genus is a fast-growing plant and can be grown in different habitats. The evergreen nature and variegated pattern of the leaves makes the plant an integral part of the nursery industry and has a high consumer preference (Boyer *et al*., 2008). Many species of this genus are seedless, thus propagation through cutting is a good option for mass production (Poston, 2007). Generally, cuttings taken in the spring and summer root early (Lee and Tukey, 1971) and the genus *Euonymus* L. is considered an easy to root species (Hartmann *et al*., 2002).

The timing of cuttings during the year determines the concentration of rooting hormone required for rooting. Generally, 1000-3000 ppm of IBA in the spring and up to 8000 ppm of IBA in the fall is required for rooting of *Euonymus alatus*(Thunb.) Siebold in 5-7 weeks (Whitcomb, 1978). However, studies on the influence of elevated CO_2 on *Euonymus* propagation has not been reported. Thus, this study was conducted with the objective of studying how elevated CO_2 concentrations affects the rooting of *Euonymus* and how CO_2 interacts with exogenous application of rooting hormone.

Materials and methods

Plant materials and growth conditions: In December 2016 and January 2017, two different shipments of 6 cm cuttings of *Euonymus* 'Moonshadow' were shipped from Greenleaf Nursery Co. (Parkhill, OK). The cuttings were kept in a cooler overnight and the next day cuttings were inserted into 5.08 cm × 5.71 cm × 8.25 cm flats (Johny's selected seed, Winslow, ME). The flats were filled with a 1:1 perlite and vermiculite mixture. About 2-3 cm of each cutting was dipped in respective rooting hormone treatments for 15 seconds and placed in media. The flats were placed on mist benches and a timer was set to turn on the system every 16 minutes for 8 seconds. In the greenhouse, day/night temperature was set at 21 ˚C/18 ˚C, respectively. Both batch of cuttings were left for rooting for 45 days and root parameters were measured.

Experimental setup and treatments: Rooting of 'Moonshdow' stem cuttingwas studied in a replicated experiment conducted in the Department of Horticulture and Landscape Architecture research greenhouse at Oklahoma State University, Stillwater, OK. Cuttings were grown in a split-plot design in which, two identical greenhouses were used. One of the greenhouse was supplemented with an average of 800 ppm of CO_2 (Fig. 1). A CO_2 generator (Johnson Gas Appliance Company, Cedar Rapids, IA) was used for CO_2 generation and was monitored by CO_2 Monitor (FLIR Commercial System Inc., Nashua, NH). The CO_2 generator was manually set to produce around 800 ppm of CO_2 by burning natural gas. The generator was set to turn on from 6:00 h to 14:00 h throughout the growing cycle.

In addition to the CO_2 treatment, the effect of different concentrations of rooting hormone (Dip'N Grow Inc., Clackamas, OR) was used in rooting. The product consists of 1% Indole-3-Butyric Acid (IBA) and 0.5% Naphthaleneacetic acid (NAA). Control, 1000, 3000, and 5000 ppm concentration of rooting hormone was applied to the basal 3-5 cm part of the cuttings. In each greenhouse, four replications of all treatments were made, and each treatment had 12 samples of cuttings.

Measurements and statistical analyses: All measurements were made from cuttings that had developed roots. Rooting percentage, root number, length of roots (average of two longest roots), root diameter, and dry weight of roots were measured. For dry weight, roots were harvested, washed, and then dried at 60 ˚C for 72 hours. All data were subjected to two-way Analysis of Variance using SAS (Statistical Analysis System) version 9.4 (SAS Institute, Cary, NC). The two ways interaction between CO_2 and different concentrations of rooting hormone was studied in two different sets of cuttings. Means were computed using PROC MIXED and pdmix800 macro program was used for mean separation between the treatments. In the case where interactions were found insignificant, means of the main effect were computed at the 0.05 level of significance.

Results

Interaction of CO_2 x Rooting Hormone was significant for most of the parameters except rooting percent and root diameter (Table 1). There was no significant difference in rooting percentage between ambient and elevated CO_2 as well as rooting hormone treatments (data not shown). Root number decreased with increasing level of CO_2 (Table 2). The cuttings placed in 5000 ppm of rooting hormone in ambient CO_2 had a significantly greater number of roots when compared to the elevated CO_2 condition. Root numbers in ambient condition were greater (27.8%) when compared to the elevated condition in 5000 ppm rooting hormone treatment. However, root number for 3000 ppm treatment in both ambient and elevated CO_2 and 5000 ppm treatment in elevated CO_2 were statistically similar, but lower than 5000 ppm rooting hormone treatment at the ambient CO_2 condition. However, for the control and 1000 ppm rooting hormone treatment, the difference in root number was similar and was the lowest. Root length was greater in the 5000 ppm rooting hormone treatment at ambient CO_2 (Table 2). However, the values for root length was statistically similar to all other treatments except the control treatment at ambient CO_2 condition. The greatest root length of 2.3 cm and smallest root length of 1.6 cm was measured in ambient CO_2 in 5000 ppm and control treatments, respectively. Similarly, root dry weight also increased with increasing concentration of rooting hormone. Cuttings treated with 5000 ppm rooting hormone in both CO_2 treatments and cuttings with 3000 ppm rooting hormone under elevated CO_2 had the highest root dry weight. Root dry weight was nearly 200% greater in the 5000 ppm treatment when compared with the control in ambient CO_2 condition. The control and 1000 ppm treatment had the smallest root dry weight and were statistically similar in both ambient and elevated CO_2 conditions.

Table 1. Analysis of variance (ANOVA) showing the effect of CO_2 (ambient at 400 ppm and elevated at an average of 800 ppm) and Dip'N Grow rooting hormone (control, 1000, 3000, and 5000 ppm) on different root parameters of *Euonymus* 'Moonshadow'

Effect	Rooting %	Root number	Root length	Root diameter	Root dry weight
CO_2	ns^z	ns	ns	ns	ns
Rooting hormone	ns	***	***	***	***
CO_2 × Rooting hormone	ns	*	*	ns	*

^{z}*, *** indicate the level of significance at $P < 0.05$ and $P < 0.0001$, respectively and ns indicates that the treatments are not significant.

Table 2. Effect of CO_2 (ambient at 400 ppm and elevated at an average of 800 ppm) and Dip'N Grow rooting hormone (control, 1000, 3000, and 5000 ppm) on different root parameters of *Euonymus* 'Moonshadow'

Hormone	Root number		Root length (cm)		Root dry weight (g)	
(ppm)	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Control	12.9d^z	11.8d	1.6b	1.8ab	0.008d	0.008d
1000	14.8d	19.1cd	1.9ab	1.8ab	0.010cd	0.014bcd
3000	23.6bc	24.3bc	2.1ab	1.9ab	0.016bc	0.020ab
5000	35.4a	27.7b	2.3a	1.9ab	0.024a	0.020ab

^{z}Means (n=96) within a parameter followed by same letters are not significantly different at $P \leq 0.05$.

Rooting hormone treatments were significantly different as a main effect for root diameter. The root diameter increased with increasing concentration of rooting hormone and was greatest with 3000 and 5000 ppm rooting hormone (Table 3). Cuttings under 3000 and 5000 ppm rooting hormone had a diameter of 0.11

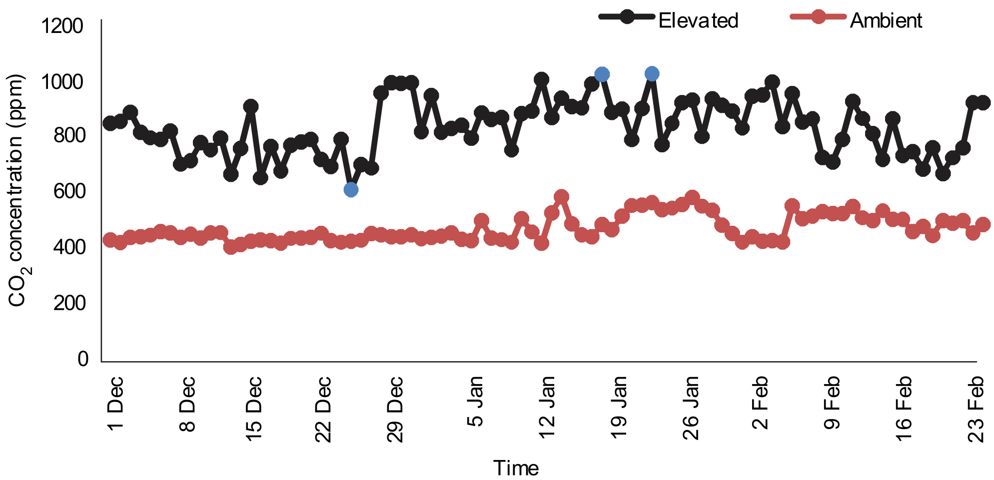

Fig. 1. Ambient and elevated CO_2 levels maintained during propagation of *Euonymus* 'Moonshadow'.

Table 3. Effect of Dip'N Grow rooting hormone (control, 1000, 3000, and 5000 ppm) on root diameter of *Euonymus* 'Moonshadow'.

Rooting hormone (ppm)	Root diameter (cm)
Control	0.10b[z]
1000	0.10b
3000	0.11a
5000	0.12a

[z]Means (n=192) within a column followed by same letters are not significantly different at $P \leq 0.05$.

and 0.12 cm, respectively, and were similar. Similarly, the control and 1000 ppm rooting hormone treatments were statistically similar and had the smallest value for root diameter. Besides the measured parameters, significant difference in leaf senescence was observed between CO_2 treatments (Fig. 2). Cuttings treated with supplemental CO_2 had greater leaf senescence but the cuttings under ambient CO_2 had intact leaves and more shoot growth (data not shown).

Discussion

Both ambient and elevated CO_2 concentrations showed gradual increments in root numbers with increasing rooting hormone concentration. Root number was the greatest at the highest concentration of root hormone under ambient CO_2 condition. However, there was no significant effect of either CO_2 or rooting hormone in rooting percentage. Poston (2007) reported that *Euonymus* sp. is an easy to root species and even rooting hormone at lower concentration is sufficient to promote rooting. Swamy *et al*. (2002) reported that the response of rooting to different rooting hormone concentrations is species specific and affected by time of year cuttings were taken. Since carbohydrate level in cuttings plays a significant role in rooting response; the change in carbohydrate content is seasonal and may affect the rooting response (Davis and Porter, 1983). However, cuttings placed in elevated CO_2 had early leaf senescence, which might have affected the rate of photosynthetic accumulation in cuttings under elevated CO_2. In contrast, the leaves were intact in ambient CO_2 and may have had a greater carbohydrate level in the stem due to more leaves, which might have resulted in more roots. However, a study in rooting of *Rhododendron* 'Anna Rose Whitney' showed no relation between carbohydrates level in the stem and rooting (French, 1990). The author reported inhibition of rooting with increasing carbohydrate level, but the mechanism is still unknown. Similarly, Lee and Tukey (1971) also reported no significant difference in root number of *Euonymus alatus*'Compactus' with increasing concentration of IBA. In support of Lee and Tukey (1971), a study in rose (*Rosa hybrid* L. 'Madelon') reported no role of auxin in the promotion of rooting but reported a significant role of auxin in cell elongation. Yet, Lee and Tukey (1971) reported increased root length with increasing IBA concentration during rooting. Like our study, Bhattacharya *et al*. (1985) reported an interaction effect of CO_2 and rotting hormone for root number and root length in sweet potato (*Ipomoea batas* (L.) Lam. 'Georgia Jet'. The authors suggested that a certain balance needs to be maintained between stem carbohydrate and auxin level for root promotion.

An increase in root dry weight with higher rooting hormone concentration in both ambient and elevated CO_2 was the result of greater number and length of roots in these treatments. For

Fig. 2. Cuttings of *Euonymus* 'Moonshadow' under (A). ambient (400 ppm) and (B). elevated (at an average of 800 ppm) CO_2. Cuttings under control, 1000, 3000, and 5000 ppm of Dip'N Grow in both pictures from left to right, respectively.

each rooting hormone treatment, the dry weight was not different when compared to ambient and elevated CO_2 condition. Like our study, Patterson *et al*. (1988) in cotton (*Gossypium hirsutum* L.) and Kaushal *et al*. (1989) in black pine (*Pinus nigra* L. 'Corsicana') also reported no effect of supplemental CO_2 in root dry weight. In contrast, Laforage *et al*. (1991) reported an increase in root dry weight of raspberry by 173-245% when compared between ambient and 1600 ppm CO_2 concentration. Although leaf abscission was not considered as one of the measured parameters in the study, the effect was clearly visible between ambient and elevated CO_2 condition in our study (Fig. 2). French (1989) reported similar leaf senescence in *Rhododendron* 'Mortha Isaacson' when propagated in the fall and only 5% of leaves were intact in cuttings misted with 1200 ppm of CO_2 solution but more than 50% were intact in case of ambient condition. Application of supplemental CO_2 in sunflower (*Helianthus annus* L.) promoted ethylene production from plant tissue (Dhawan *et al*., 1981). Since, ethylene is responsible in leaf senescence, the increased ethylene might have resulted in leaf abscission under elevated CO_2 condition (French, 1989). However, the author also mentioned that ethylene production in elevated CO_2 might affect leaf abscission but may promote rooting A similar negative effect could have happened in our supplemental CO_2 study too, which resulted in leaf senescence but no difference in rooting percentage between CO_2 treatments. In the future, more studies are needed with multiple CO_2 levels, different types of rooting hormone, and at different concentrations in multiple species to fully understand the effect on root development.

Elevated CO_2 did not show positive effect in rooting of *Euonymus* 'Moonshadow'. Although many studies have reported positive effect of elevated CO_2, the response is species specific. Application of rooting hormone improved root numbers in 'Moonshadow' and cuttings had a well-established root system with 3000 or 5000 ppm hormone. Supplemental CO_2 could not be recommended for rooting of *Euonymus* 'Moonshadow'; however, future research in supplemental CO_2 should consider the interaction with various environmental and cultural factors, which might result in increased rooting.

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